

Mating Scheme For Production Of HbA Replacement Mice

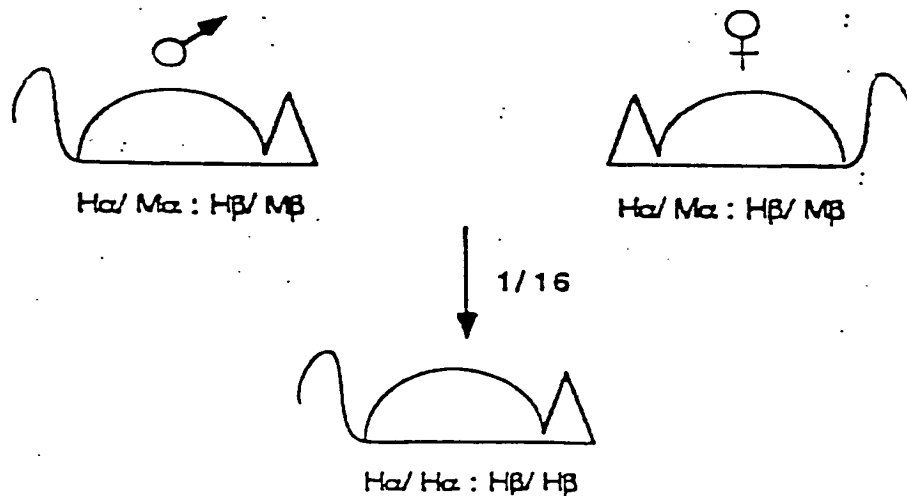
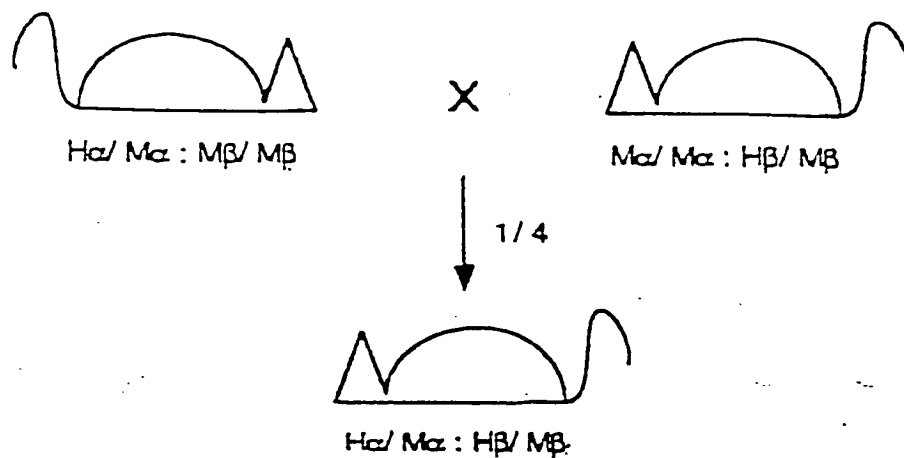
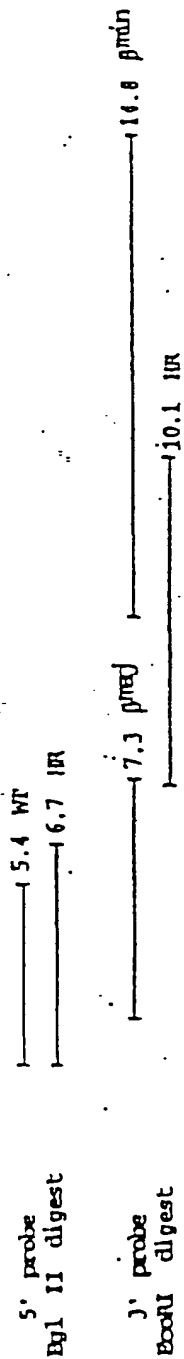
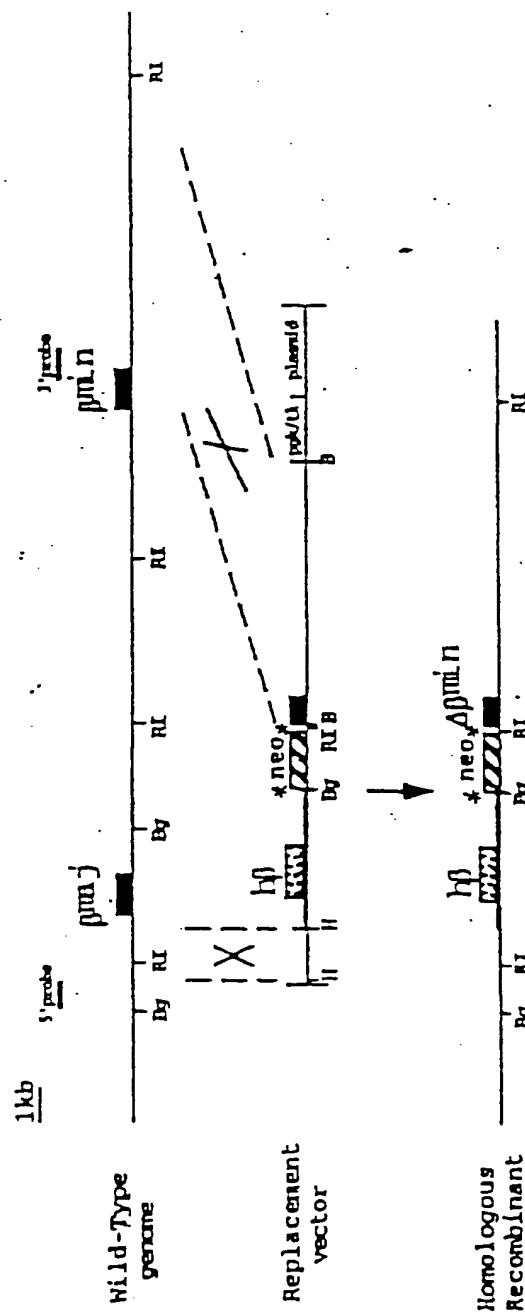
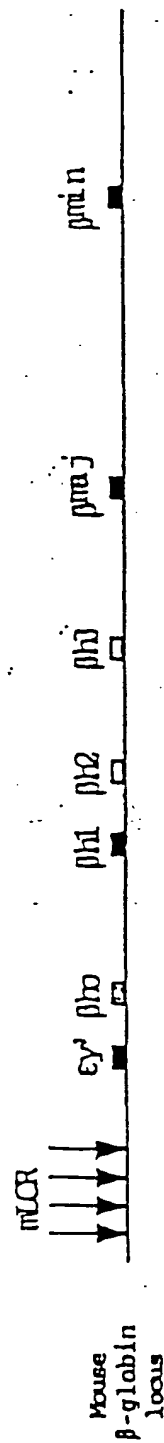


FIG. 1

FIG. 2

Mouse β KO/human β replacement



The diagram illustrates the structure of the human and mouse β -globin loci and various gene replacement constructs. The loci are represented as horizontal lines with genes and regulatory elements indicated by black boxes. The genes are labeled as ϵ , γ , δ , and β . The regulatory elements include the LCR (Locus Control Region) and enhancers (e.g., ϵ enhancer, γ enhancer, δ enhancer). The constructs are labeled A through I, representing different experimental models for gene replacement.

- A**: Mouse β -Globin Locus. Shows the arrangement of genes ϵ , γ , δ , and β with their respective enhancers and the LCR.
- B**: Hprt Tag. Shows the Hprt gene flanked by the LCR and enhancers.
- C**: Human β^s Replacement. Shows the human β^s gene flanked by the LCR and enhancers.
- D**: Human $\gamma\beta^s$ Replacement-1. Shows the human γ and β^s genes flanked by the LCR and enhancers.
- E**: Human $\gamma\beta^s$ Replacement-2. Shows the human γ and β^s genes flanked by the LCR and enhancers.
- F**: Human LCR $\gamma\beta^s$ Replacement. Shows the human LCR and $\gamma\beta^s$ gene flanked by the LCR and enhancers.
- G**: Human $\epsilon\gamma\delta\beta^s$ Replacement. Shows the human ϵ , γ , δ , and β^s genes flanked by the LCR and enhancers.
- H**: Human LCR $\epsilon\gamma\delta\beta^s$ Replacement. Shows the human LCR and $\epsilon\gamma\delta\beta^s$ gene flanked by the LCR and enhancers.
- I**: Human LCR $\epsilon\gamma\delta\beta^s$ 3'HS1 Replacement. Shows the human LCR and $\epsilon\gamma\delta\beta^s$ gene flanked by the LCR and enhancers, with a 3'HS1 element.

The diagram illustrates the structure of the human and mouse β -globin loci and various gene replacement constructs. The human locus (A-I) and mouse locus (A) are shown with their respective genes and regulatory elements. The constructs represent different experimental models for gene replacement, including human and mouse sequences and specific gene targeting strategies.

- A** Mouse β -Globin Locus: Shows the mouse β -globin locus with genes ϵ , $\beta H10$, $\beta H11$, $\beta H12$, $\beta H13$, $\beta H14$, and $\beta H15$. The LCR is located upstream of the ϵ gene.
- B** Hprt Tag: Shows the human β -globin locus with the ϵ gene replaced by the Hprt gene. The LCR is located upstream of the ϵ gene.
- C** Human β^s Replacement: Shows the human β -globin locus with the ϵ gene replaced by the human β^s gene. The LCR is located upstream of the ϵ gene.
- D** Human $\gamma\beta^s$ Replacement-1: Shows the human β -globin locus with the ϵ gene replaced by the human $\gamma\beta^s$ gene. The LCR is located upstream of the ϵ gene.
- E** Human $\gamma\beta^s$ Replacement-2: Shows the human β -globin locus with the ϵ gene replaced by the human $\gamma\beta^s$ gene. The LCR is located upstream of the ϵ gene.
- F** Human LCR $\gamma\beta^s$ Replacement: Shows the human β -globin locus with the ϵ gene replaced by the human $\gamma\beta^s$ gene. The LCR is located upstream of the ϵ gene.
- G** Human $\epsilon\gamma\delta\beta^s$ Replacement: Shows the human β -globin locus with the ϵ gene replaced by the human $\epsilon\gamma\delta\beta^s$ gene. The LCR is located upstream of the ϵ gene.
- H** Human LCR $\epsilon\gamma\delta\beta^s$ Replacement: Shows the human β -globin locus with the ϵ gene replaced by the human $\epsilon\gamma\delta\beta^s$ gene. The LCR is located upstream of the ϵ gene.
- I** Human LCR $\epsilon\gamma\delta\beta^s$ 3'HS1 Replacement: Shows the human β -globin locus with the ϵ gene replaced by the human $\epsilon\gamma\delta\beta^s$ 3'HS1 gene. The LCR is located upstream of the ϵ gene.

Human Replacement Of The Mouse α -Globin Locus

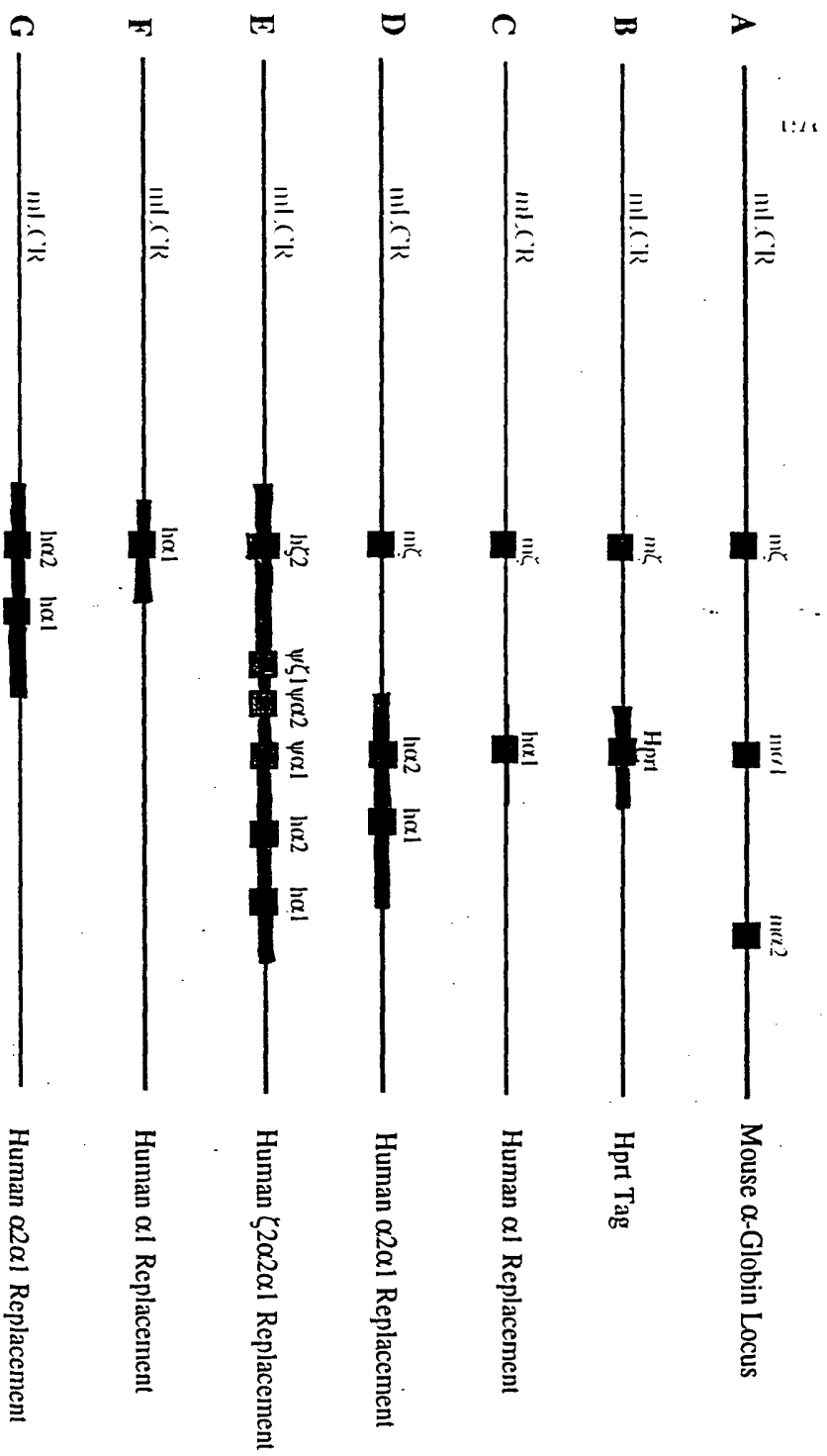


Fig. 4

Production Of Transgenic HbF \rightarrow HbA Mice
 (Doubly Homozygous For Mouse α -Globin And β -Globin Deletions)

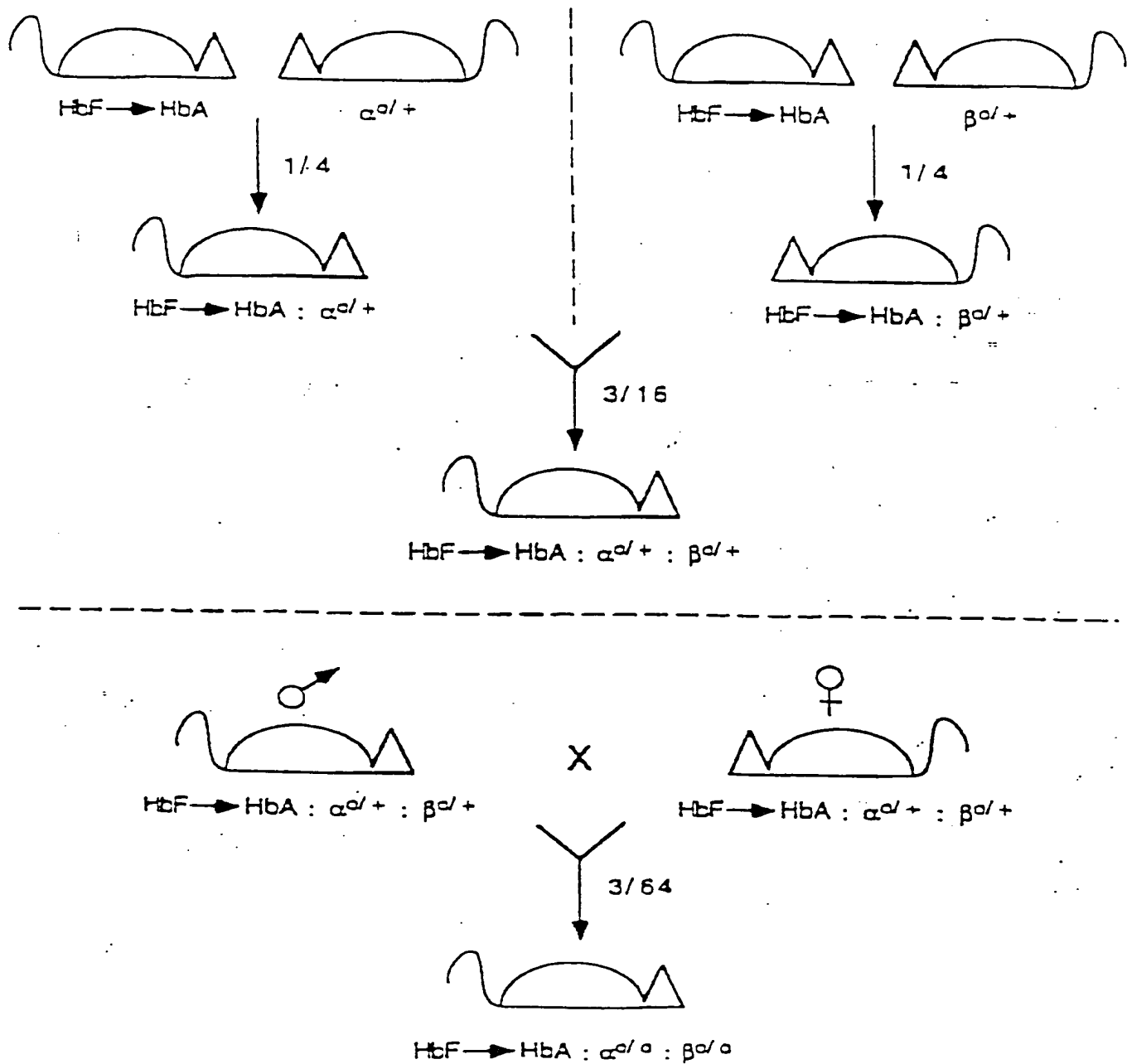
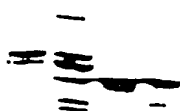


FIG. 5

Isoelectric Focusing Gel Of Transgenic Mouse Hemolysates

1 2 3 4

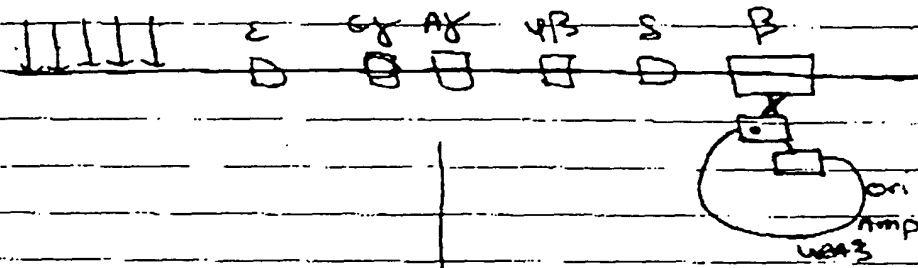


1. Mouse Control
2. HbF \rightarrow HbA : $\alpha^{+/+}$: $\beta^{+/+}$ Mouse
3. HbF \rightarrow HbA : $\alpha^{0/0}$: $\beta^{0/0}$ Mouse
4. Human AA Control

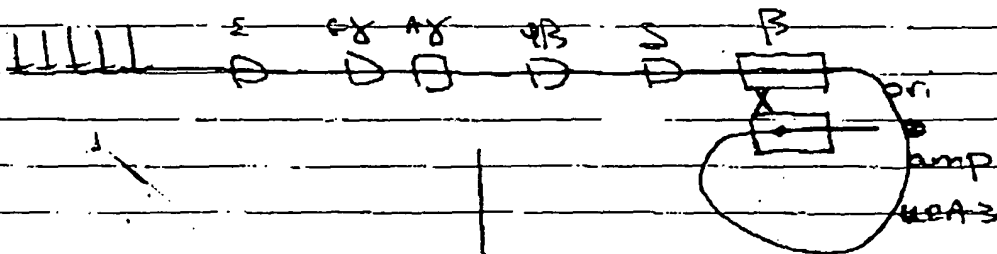
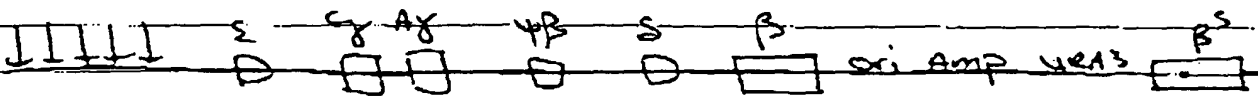
FIG. 6

FIG. 7

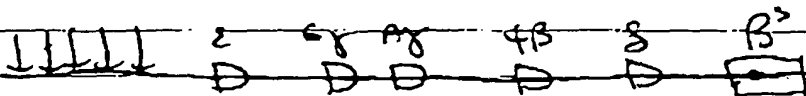
YAC



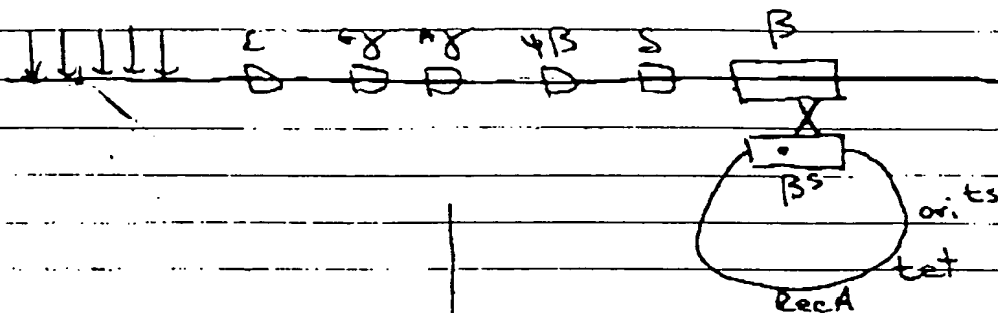
select for URA3⁺



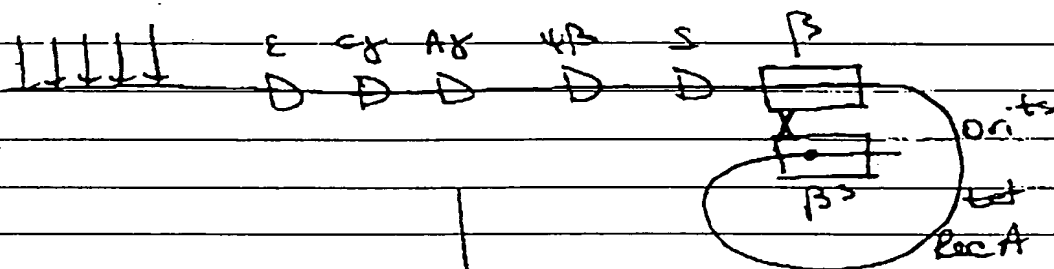
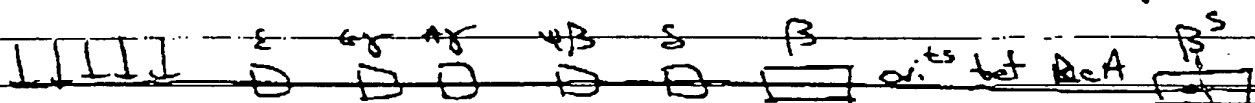
select for URA3
(5-fluoro-uracil)



BAC



select for tet^R at $43^\circ C$
(non-permissive temp for ori^{ts})



select for tet^s (Fusaric Acid)
at $37^\circ C$

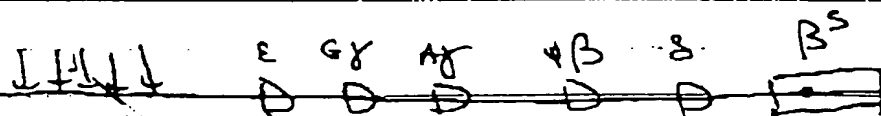


FIG. 9A

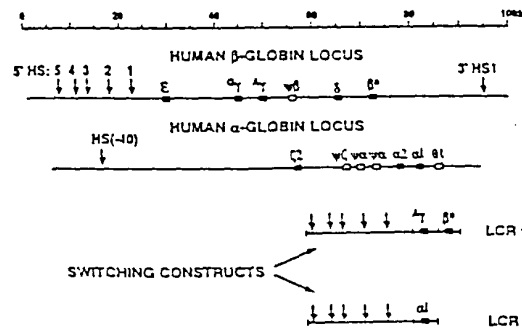


FIG. 9C

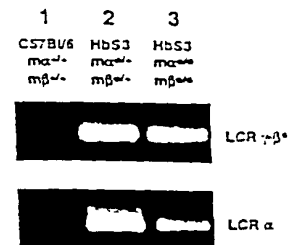
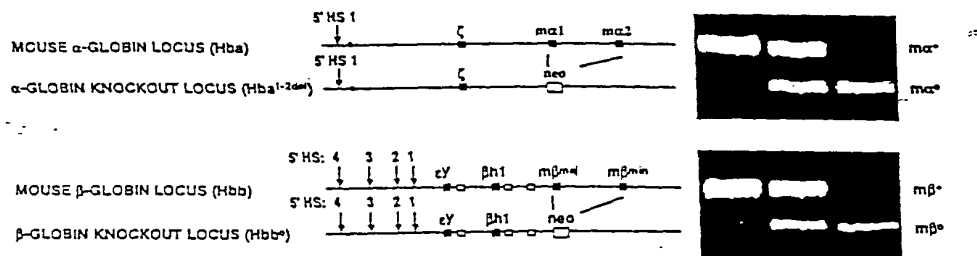


FIG. 9B



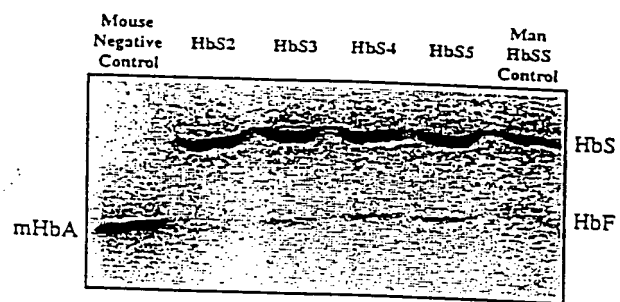


FIG. 10

FIG. 11A

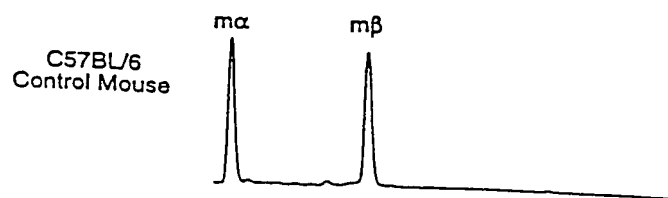


FIG. 11B

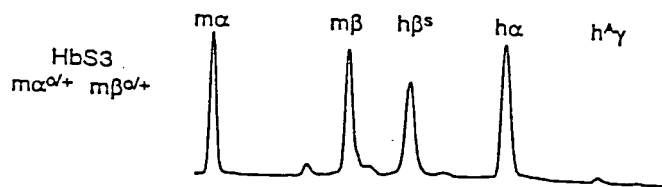


FIG. 11C

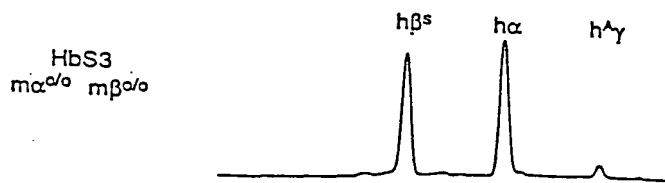
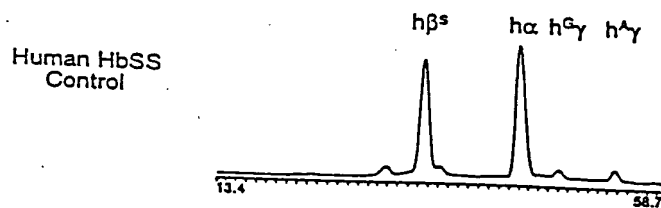


FIG. 11D



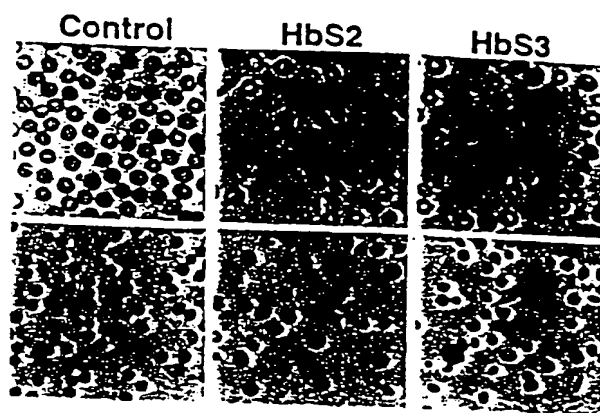


FIG. 12

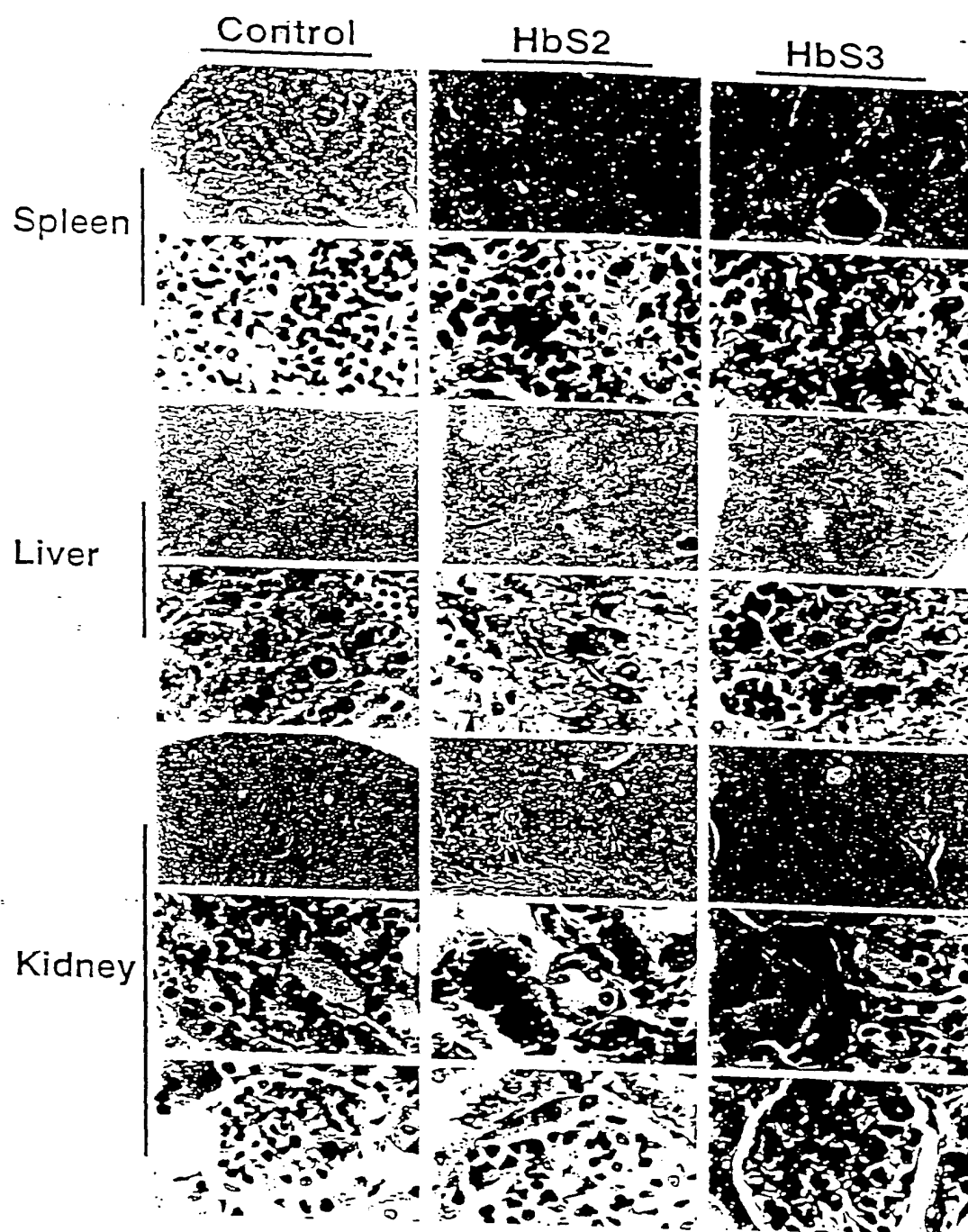


FIG. 13

FIG. 14A

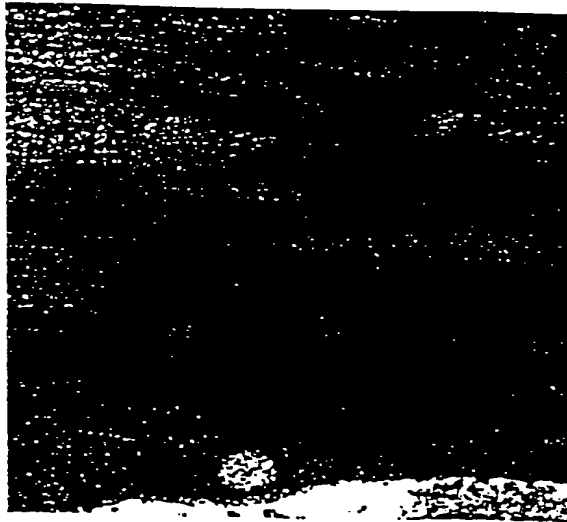


FIG. 14B



Hemoglobin Switching In HbA Mice

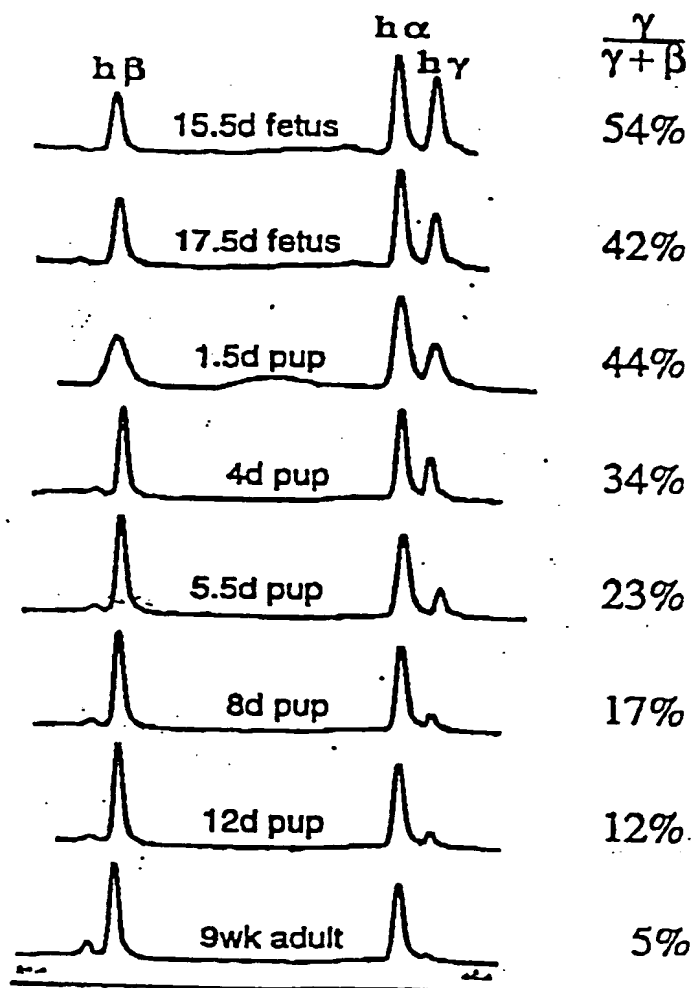


FIG. 15

**Increasing HbF Levels In HbS Mice:
Crossing The HbS 3 and HbF Lines**

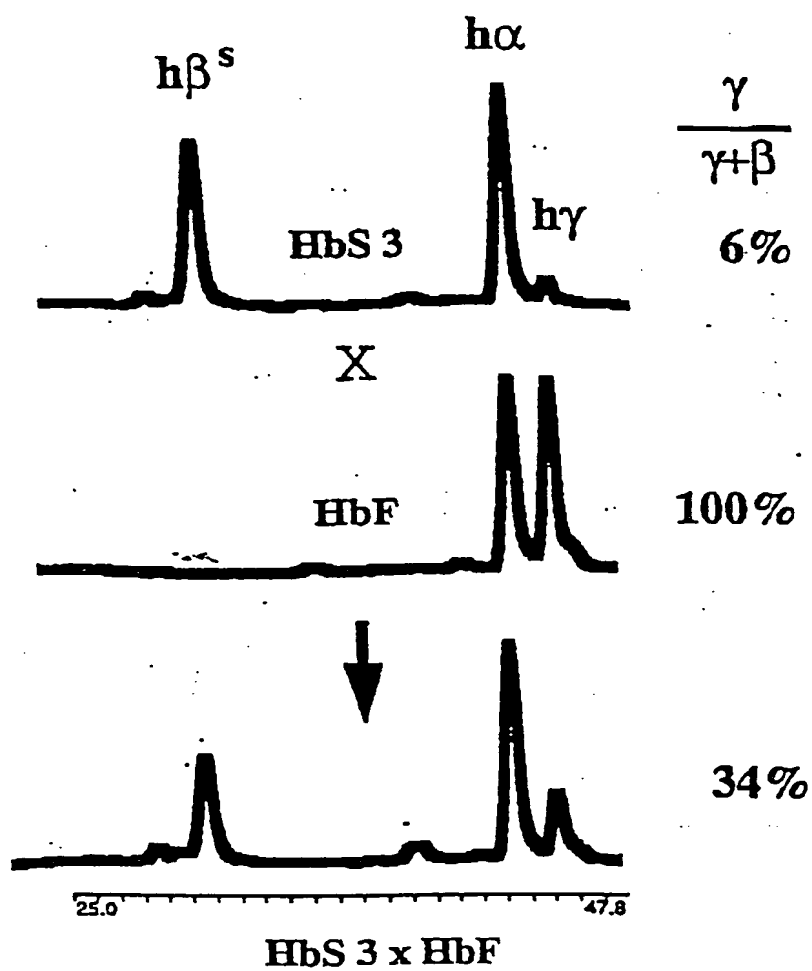


FIG. 16